



DEVICE AND METHOD FOR HIGH-THROUGHPUT
QUANTIFICATION OF mRNA FROM...
Masato Mitsuhashi
Appl. No.: 10/796,298 Atty Docket: HITACHI.055CP2
REPLACEMENT SHEET

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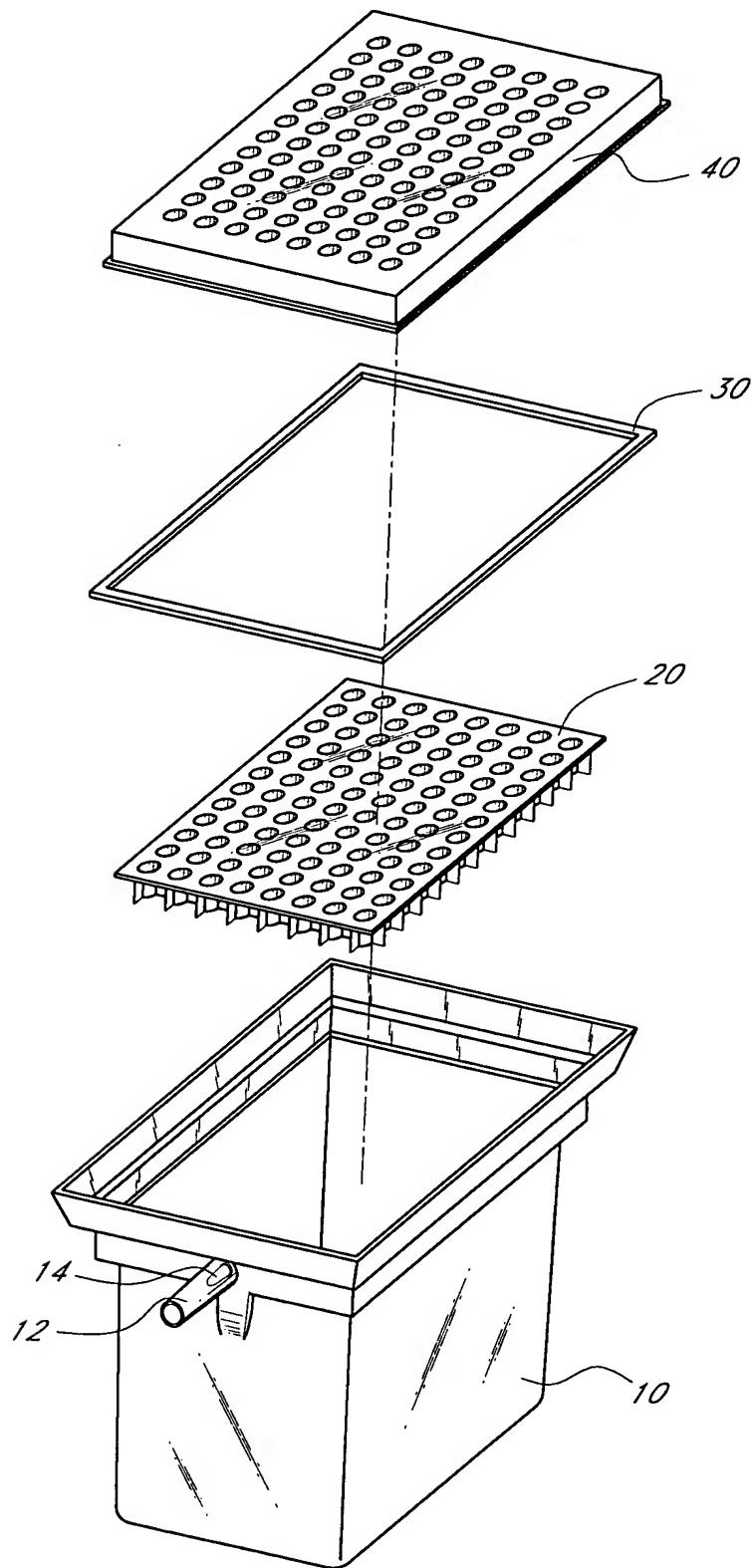


FIG. 1

*DEVICE AND METHOD FOR HIGH-THROUGHPUT
QUANTIFICATION OF mRNA FROM...*
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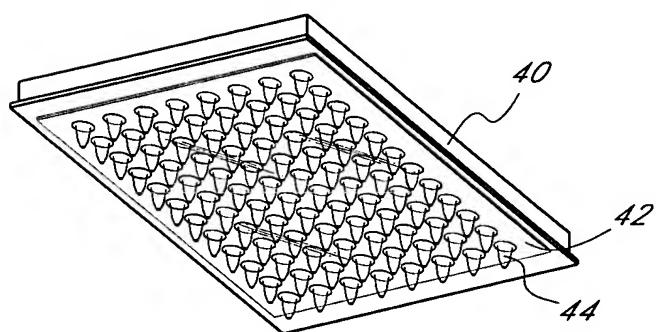


FIG. 2

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Efficiency of leukocyte trapping of fresh and frozen blood samples on filterplates

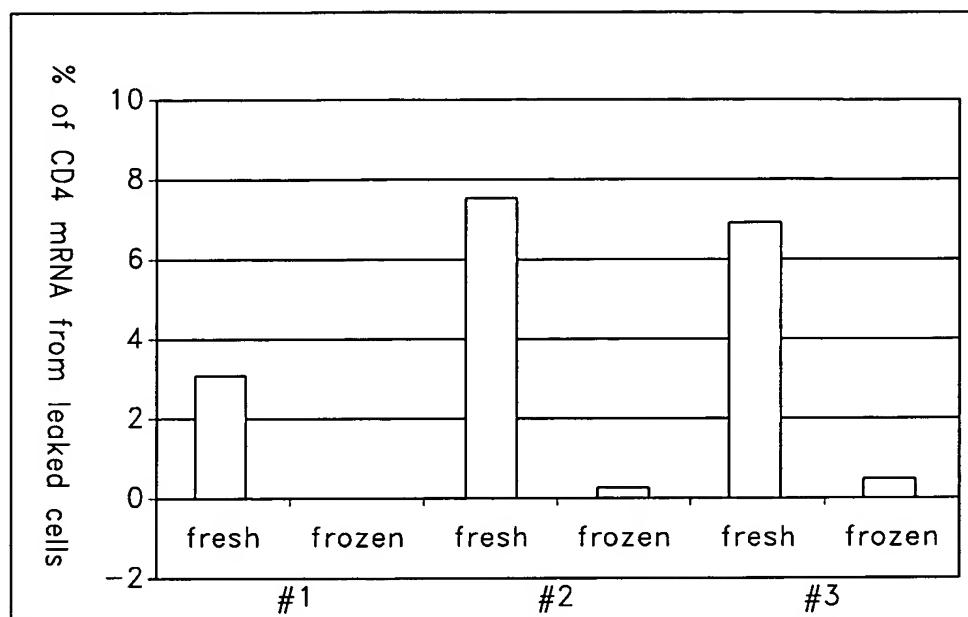


FIG. 3

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Number of washes after application of blood

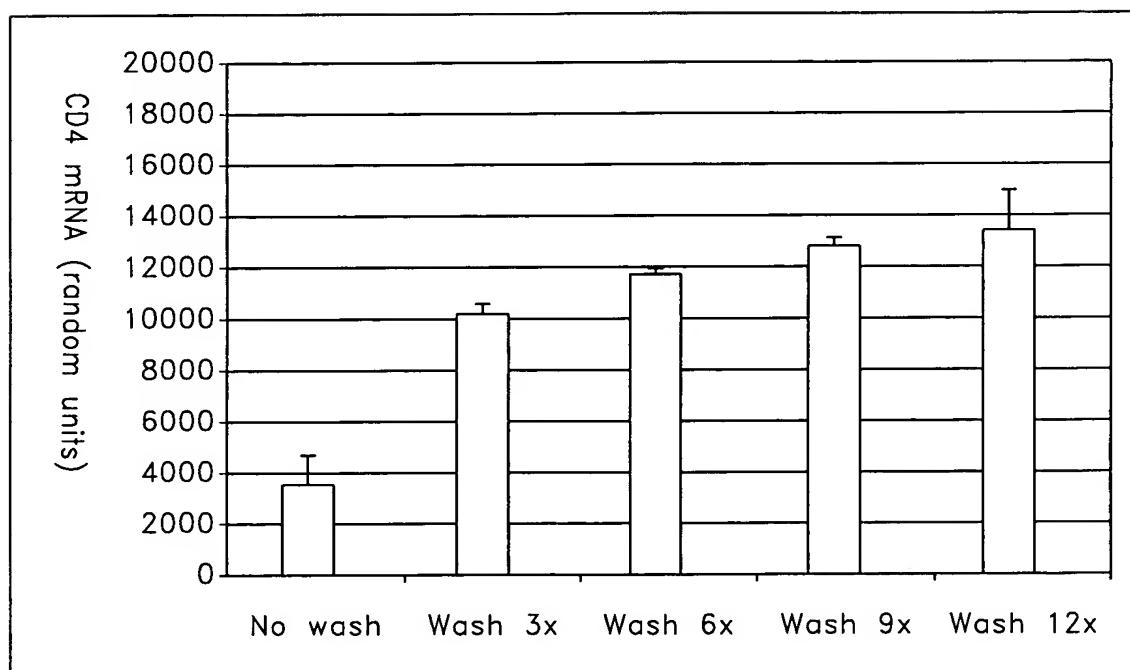


FIG. 4

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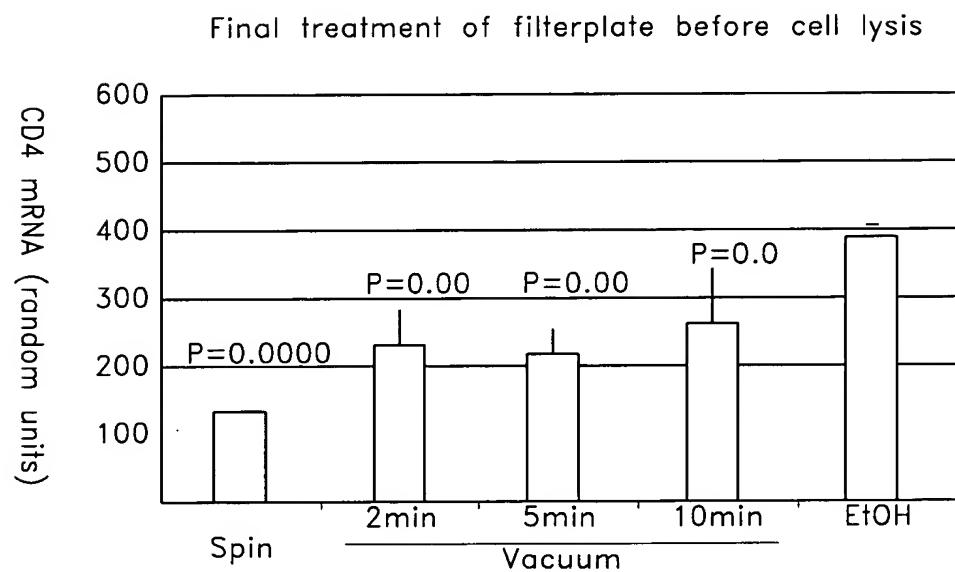


FIG. 5

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Lysis Buffer: RNase inhibition

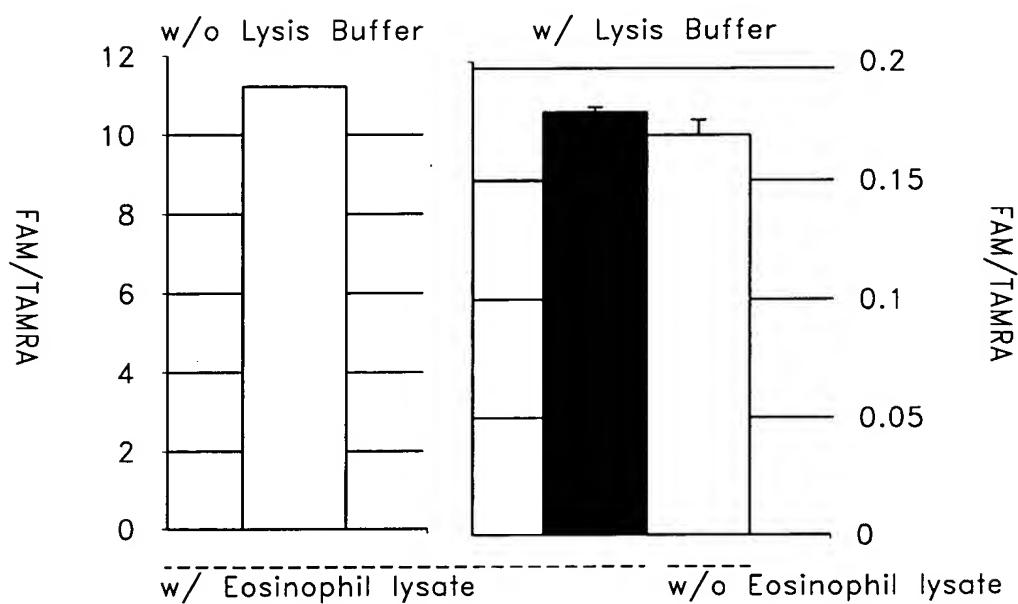


FIG. 6

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Optimal concentrations of reverse transcriptase

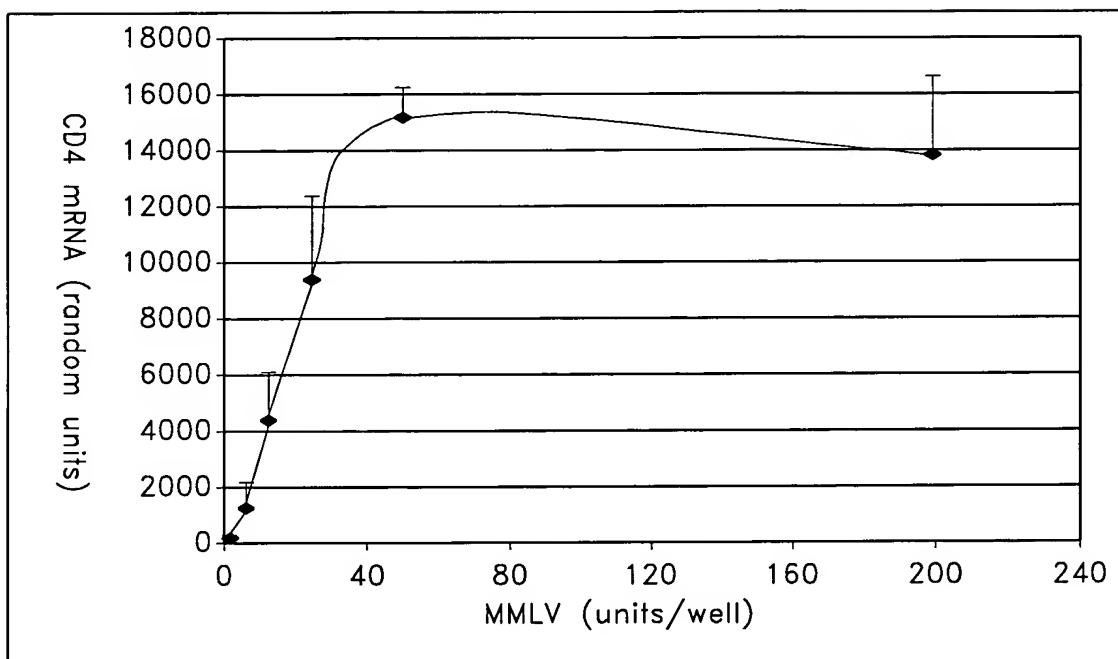


FIG. 7

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Optimal volume of cDNA for PCR

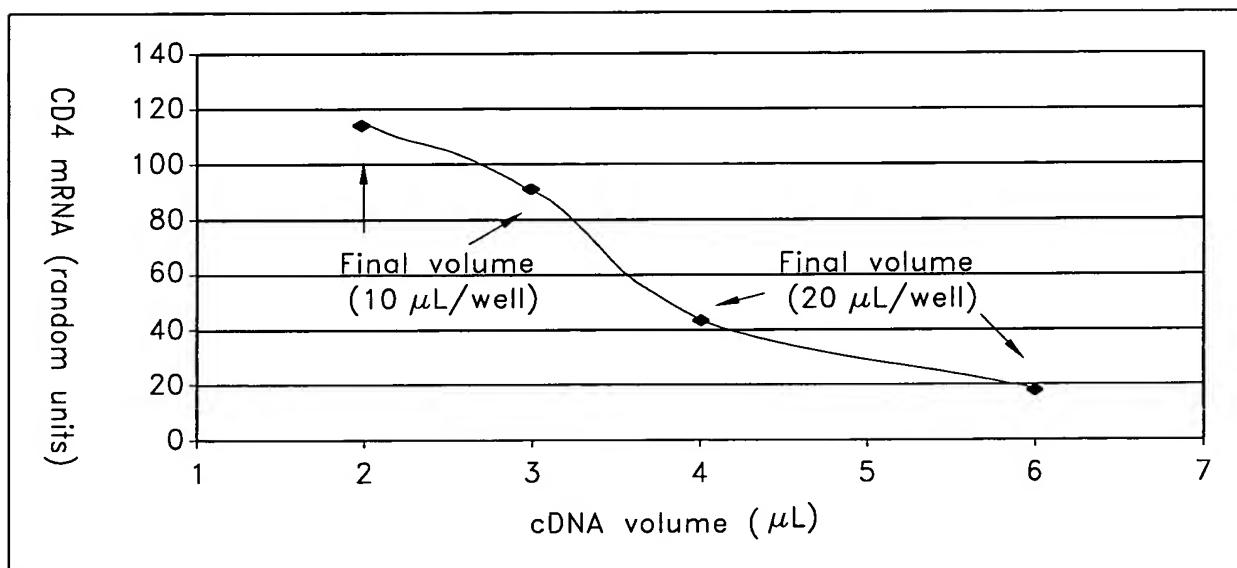


FIG. 8

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Hybridization kinetics

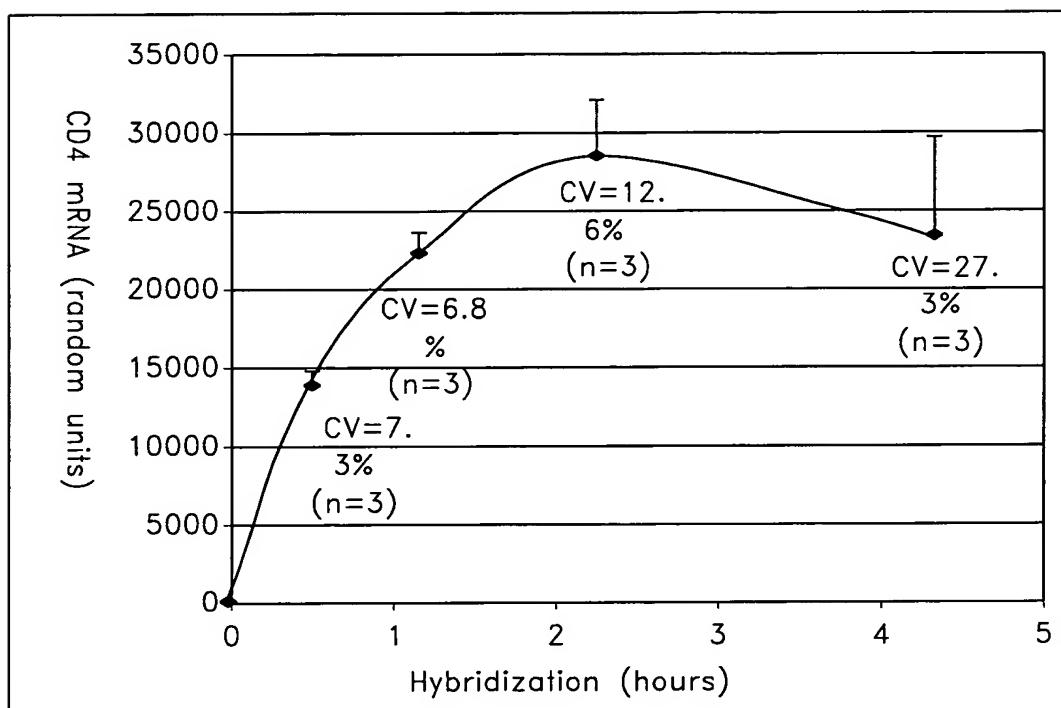


FIG. 9

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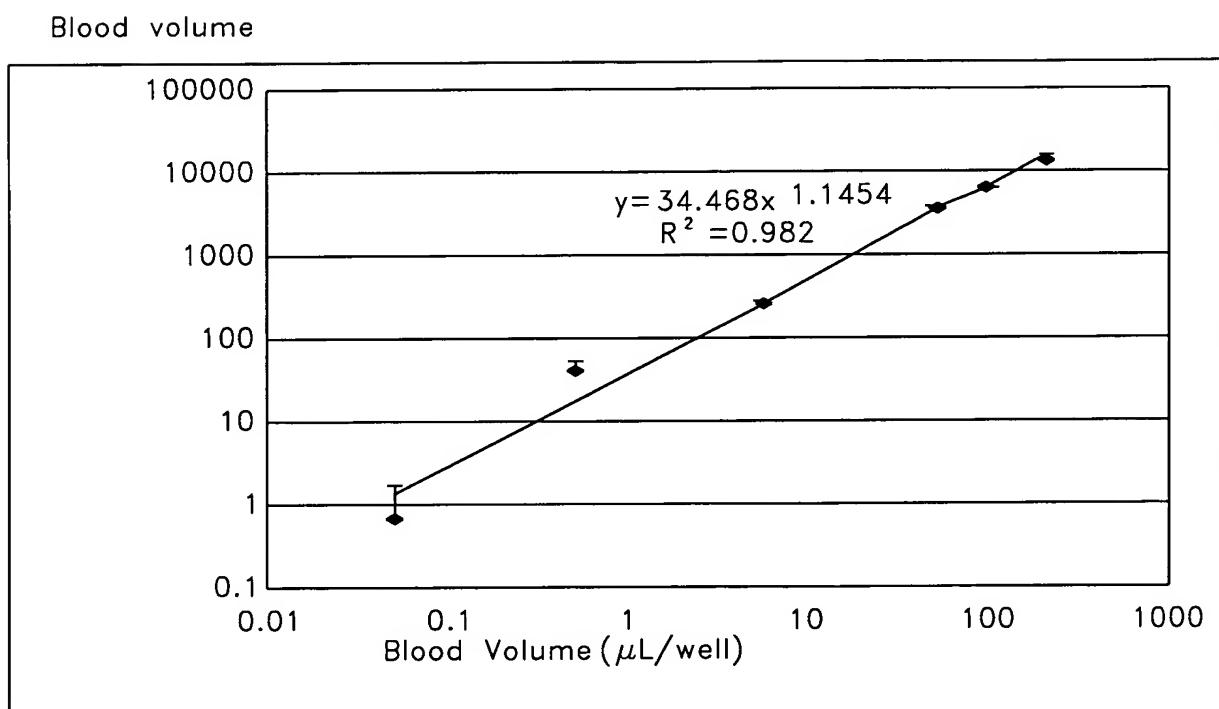


FIG. 10

Optimal guanidine thiocyanate concentration.

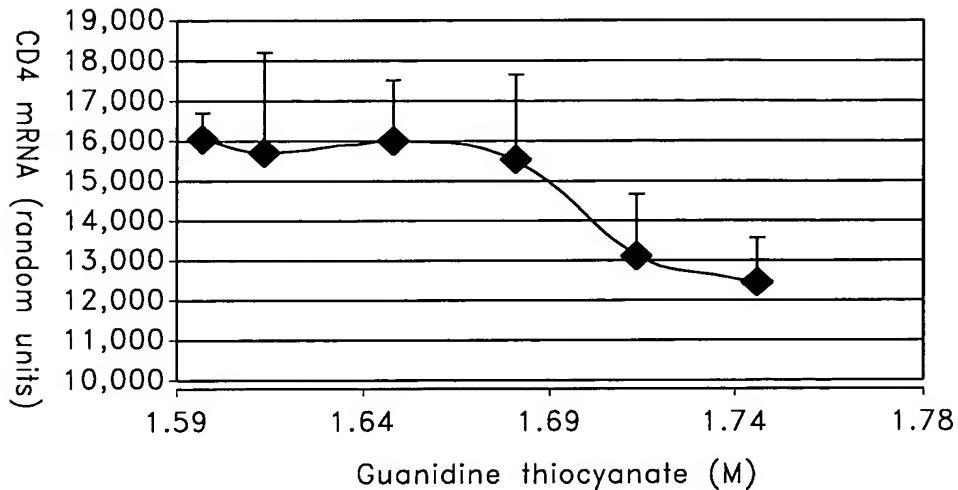


FIG. 11

Optimal proteinase K concentration.

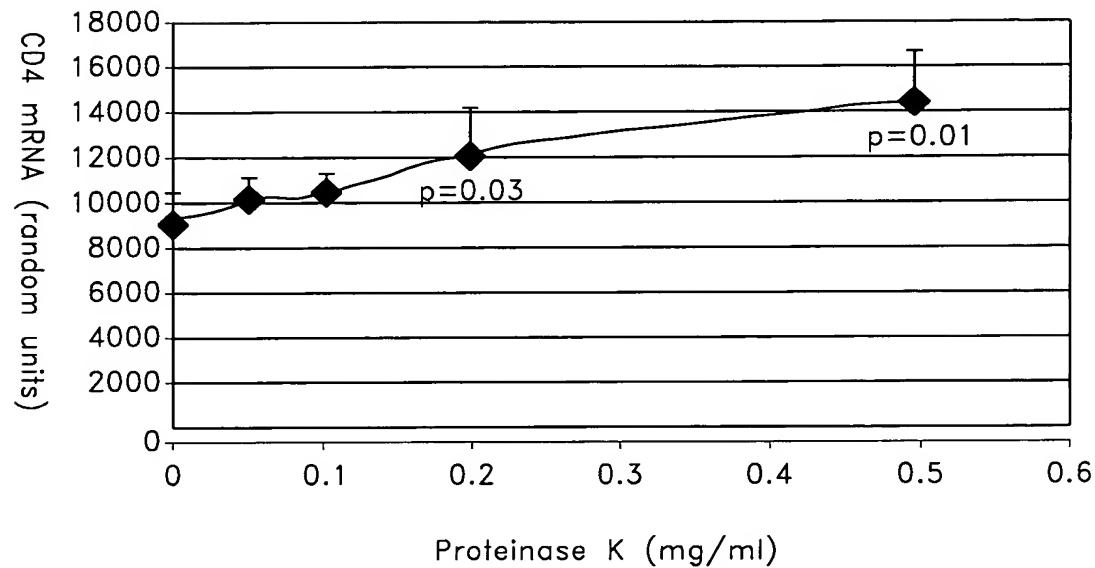


FIG. 12

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Assay Validation.

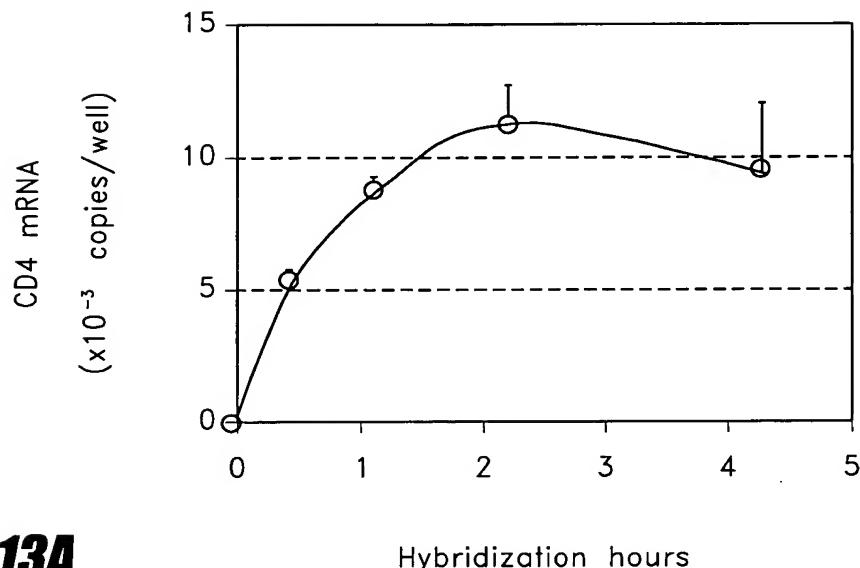


FIG. 13A

Hybridization hours

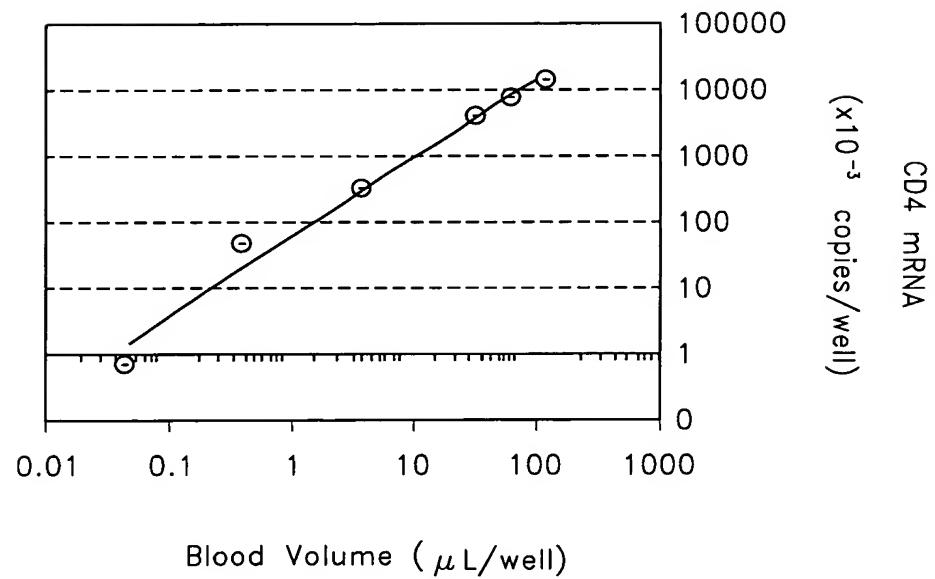


FIG. 13B

Blood Volume ($\mu\text{L}/\text{well}$)

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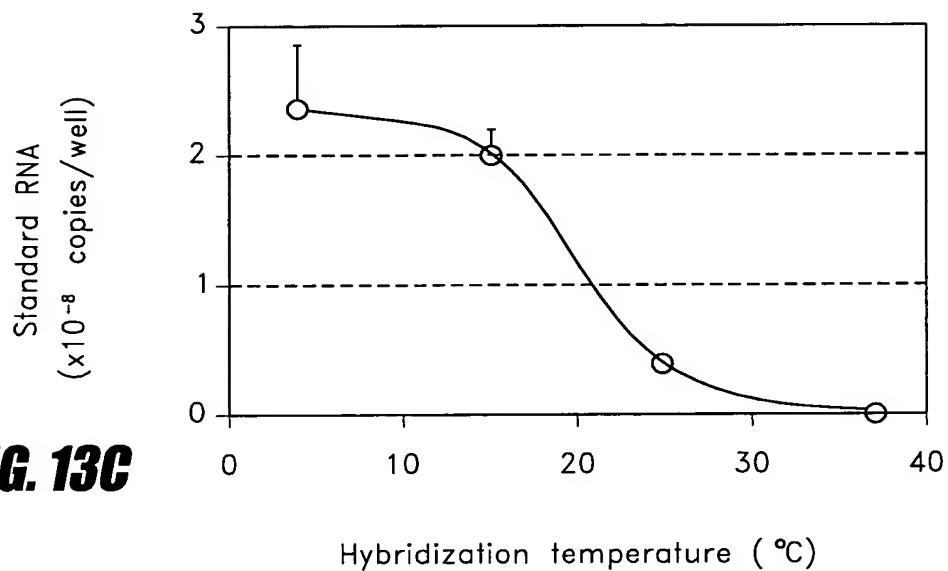


FIG. 13C

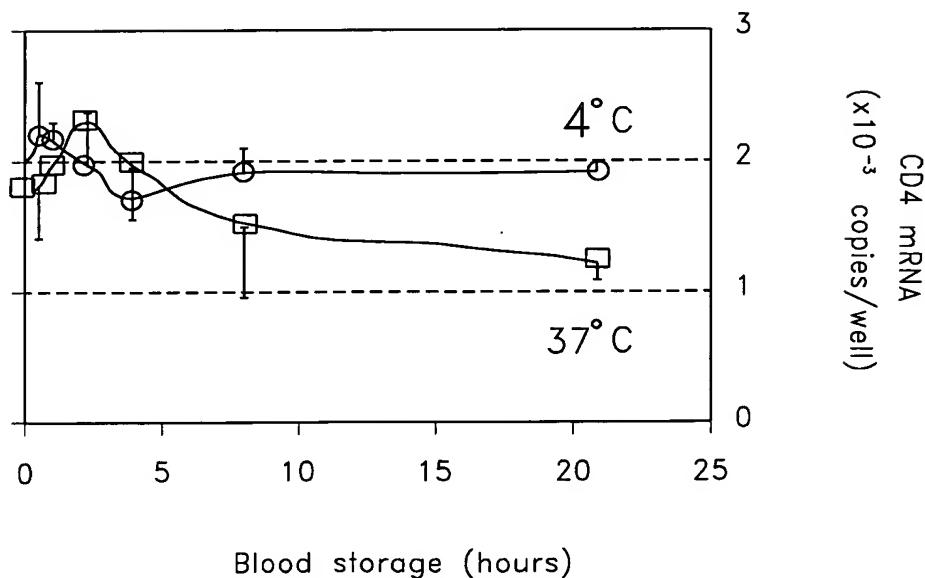


FIG. 13D

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Recovery of Synthetic Spiked RNA

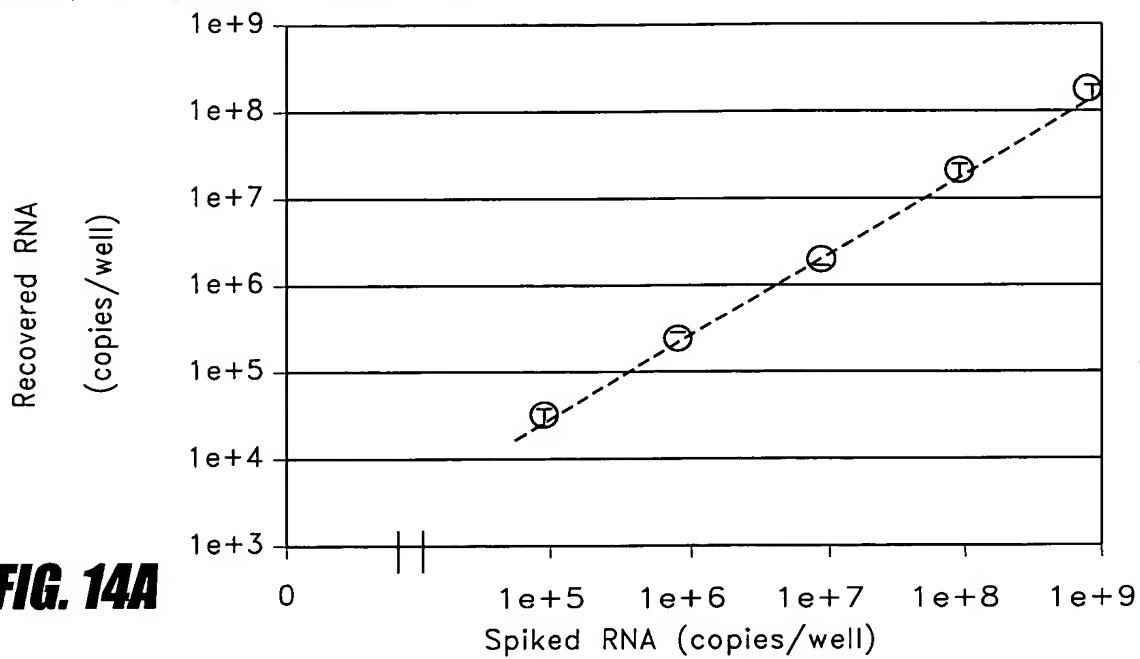


FIG. 14A

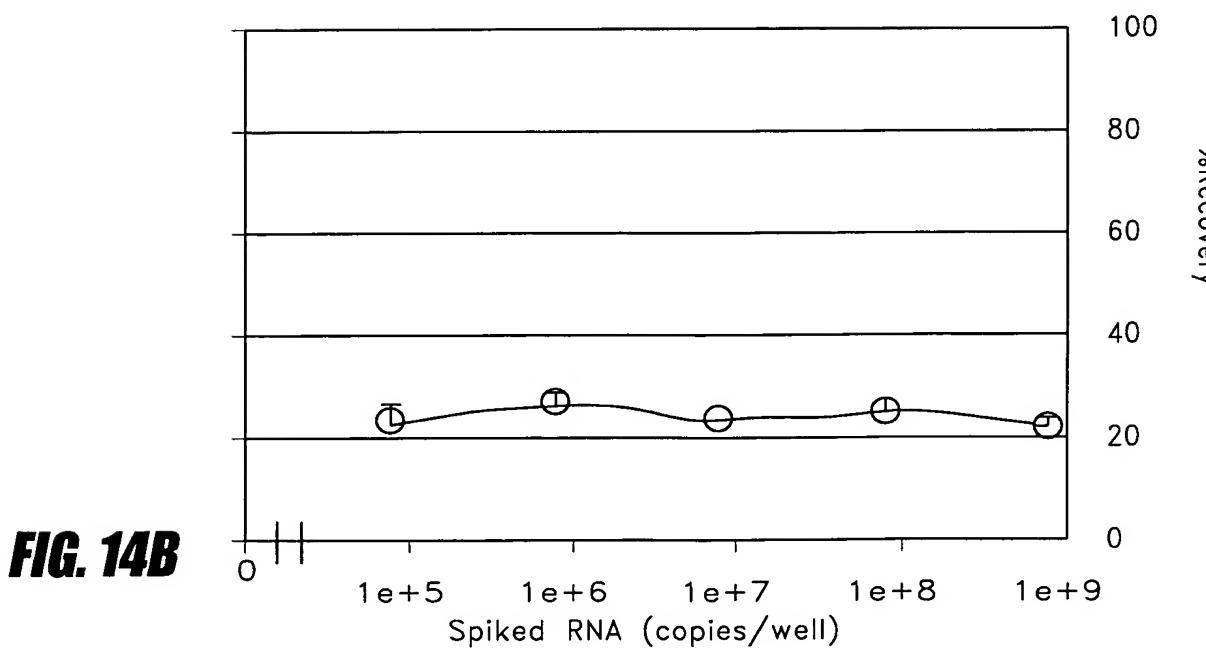


FIG. 14B

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Recovery of Synthetic Spiked RNA

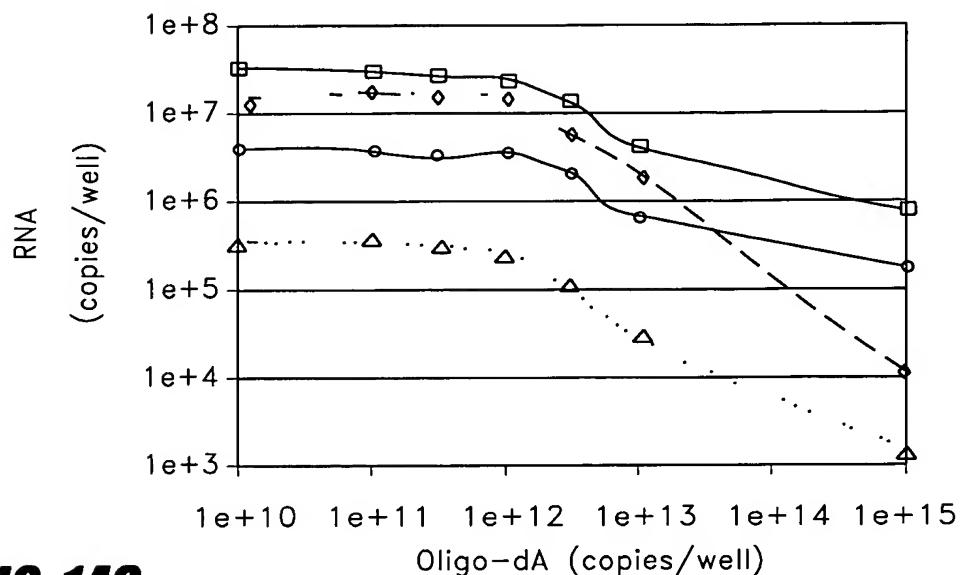


FIG. 14C

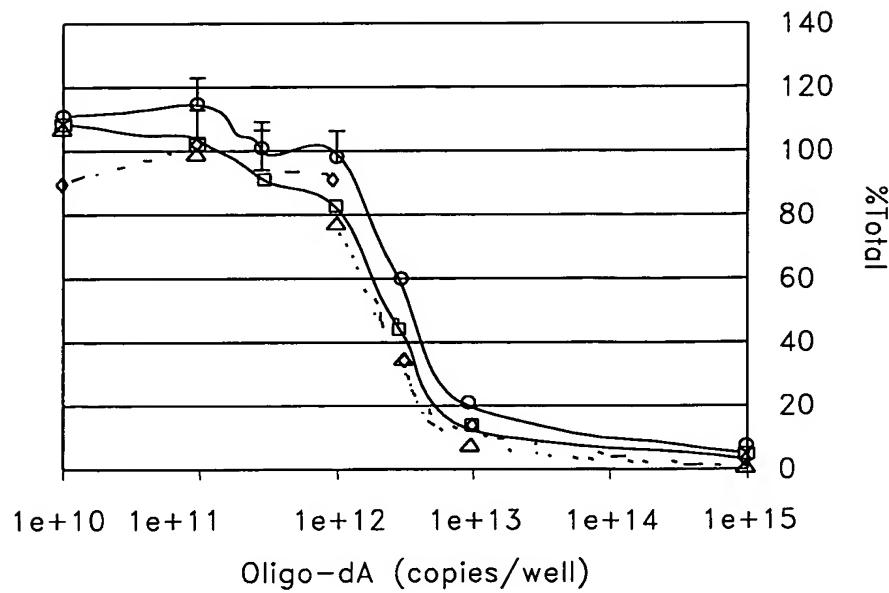


FIG. 14D

cDNA synthesis from both specific antisense primer (NNN) and immobilized oligo(dT).

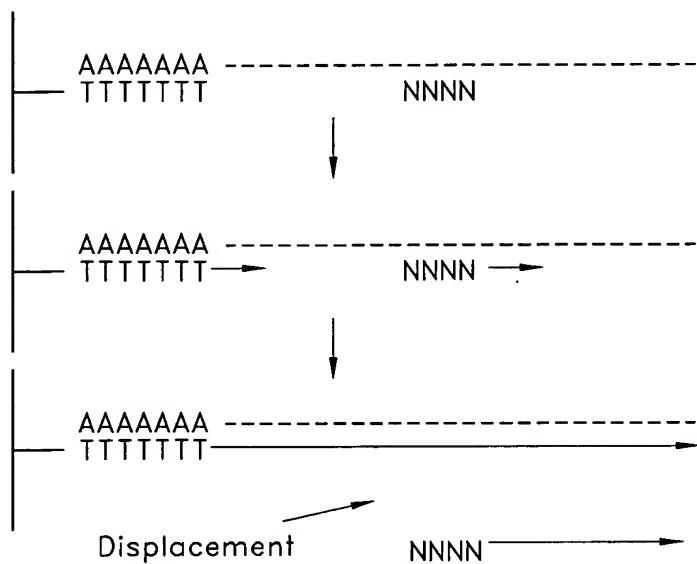


FIG. 15

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Recovery of specifically primed RNA with and without denaturization.

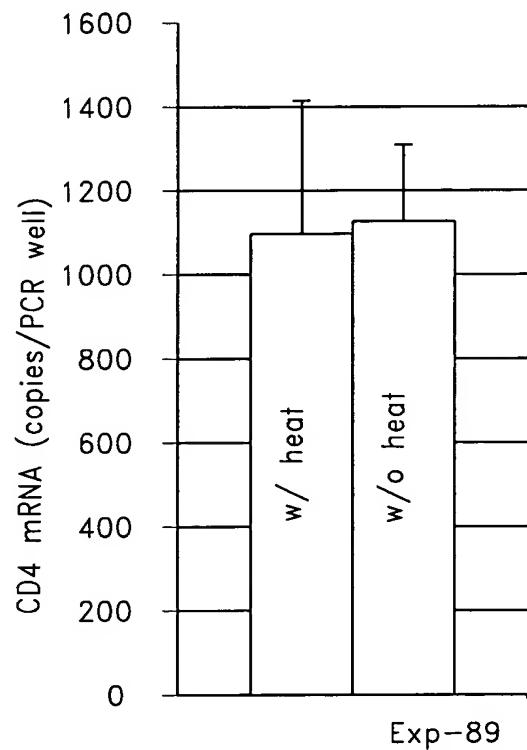


FIG. 16

Amplification of RNA with and without specific primers.

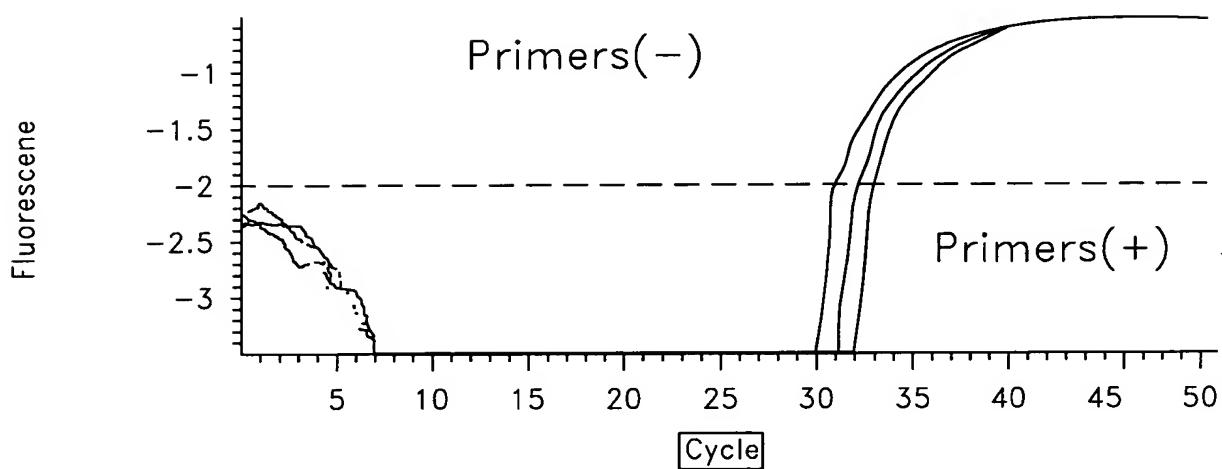


FIG. 17

mRNA quantification scheme using control RNA.

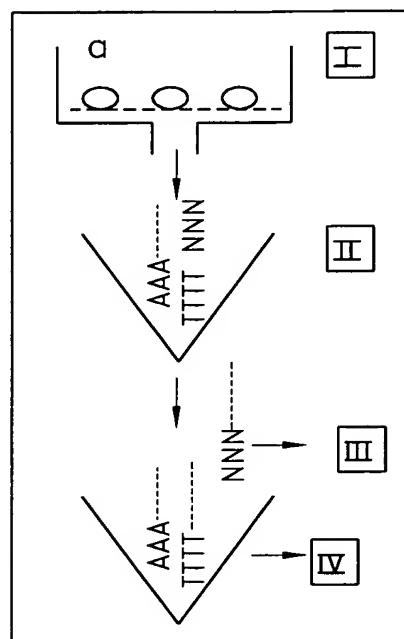


FIG.18A

mRNA quantification scheme using control RNA

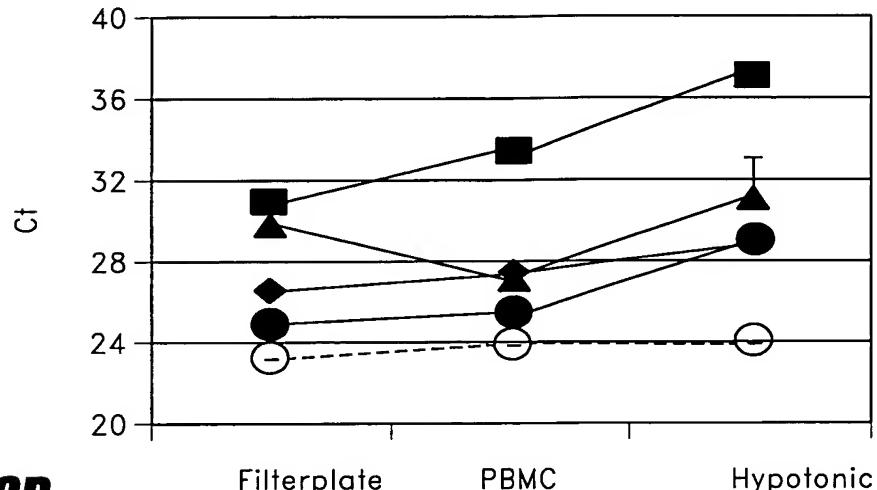


FIG. 18B

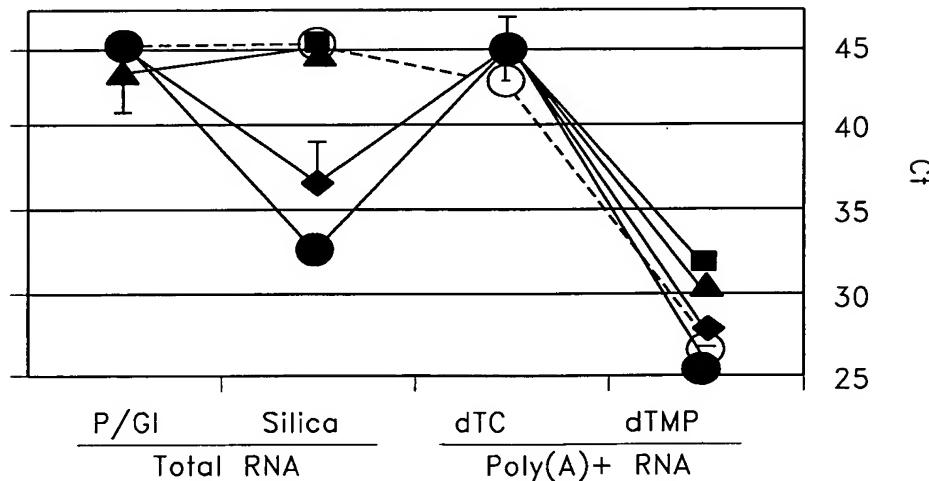


FIG. 18C

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mRNA quantification scheme using control RNA

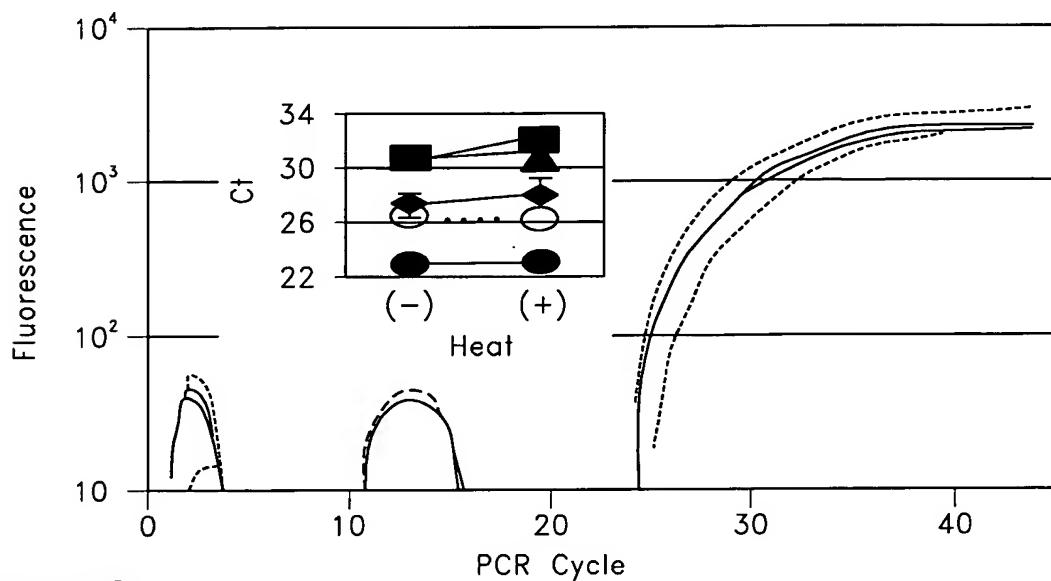


FIG. 18D

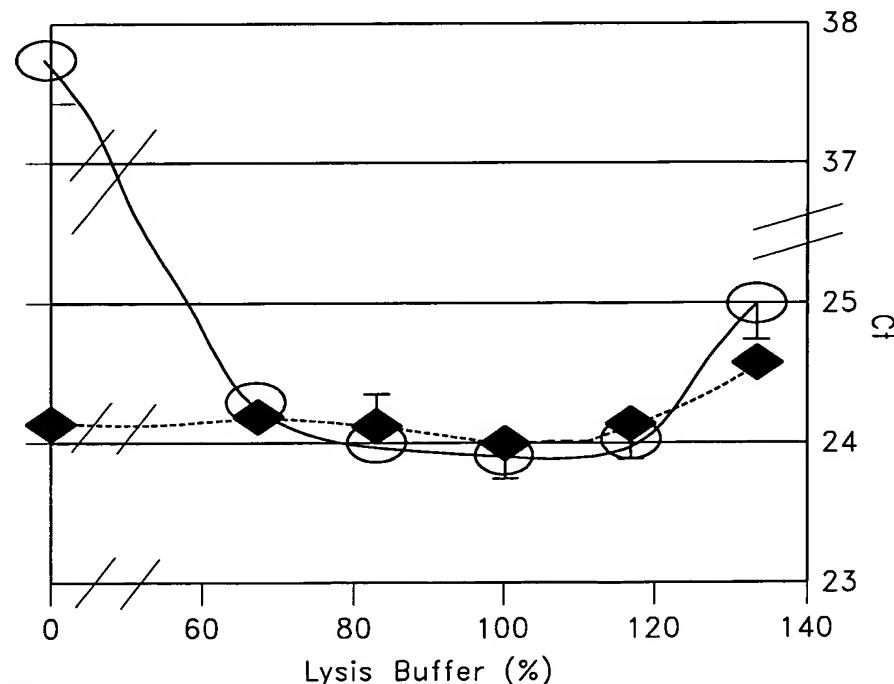


FIG. 18E

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PCR cycles.

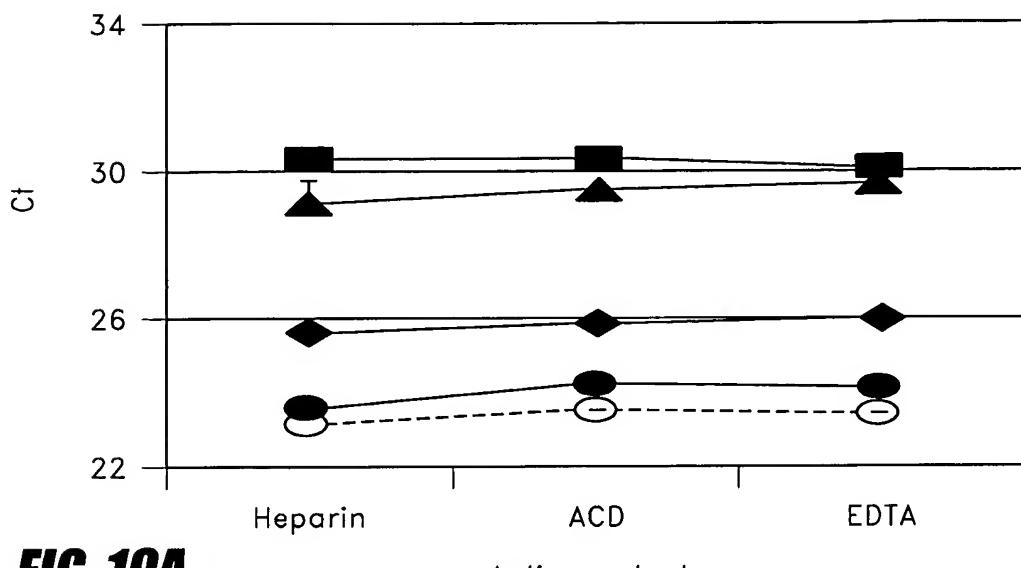


FIG. 19A

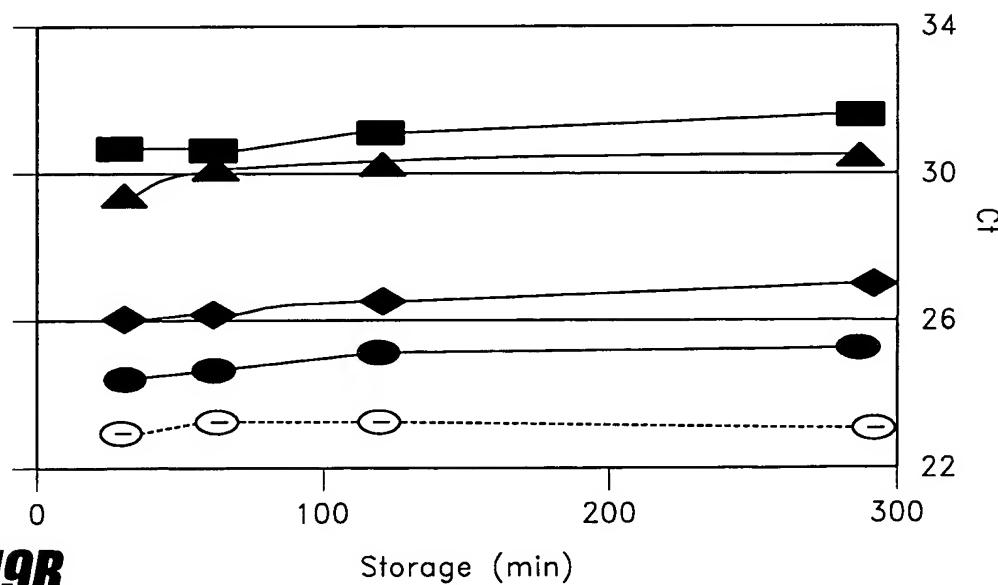


FIG. 19B

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PCR cycles.

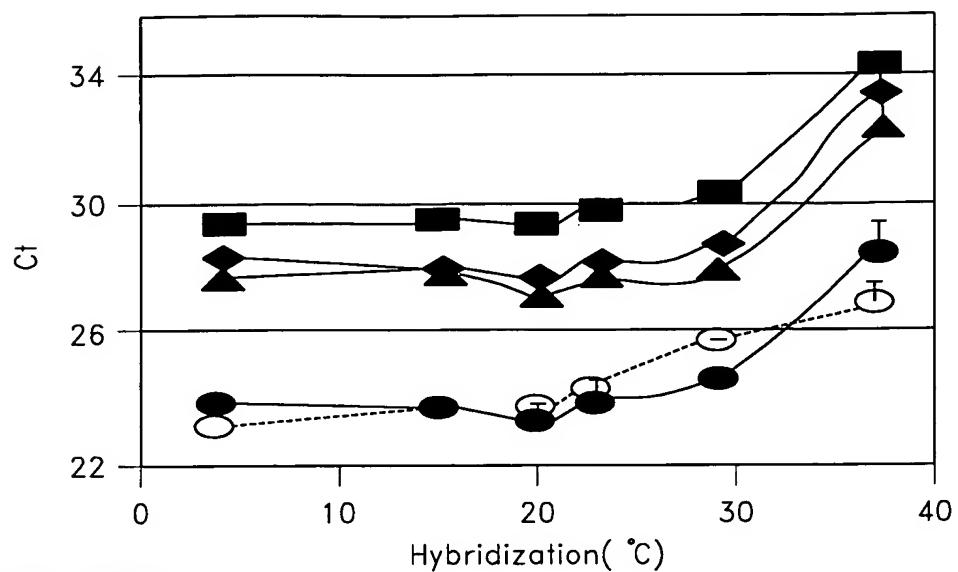


FIG. 19C

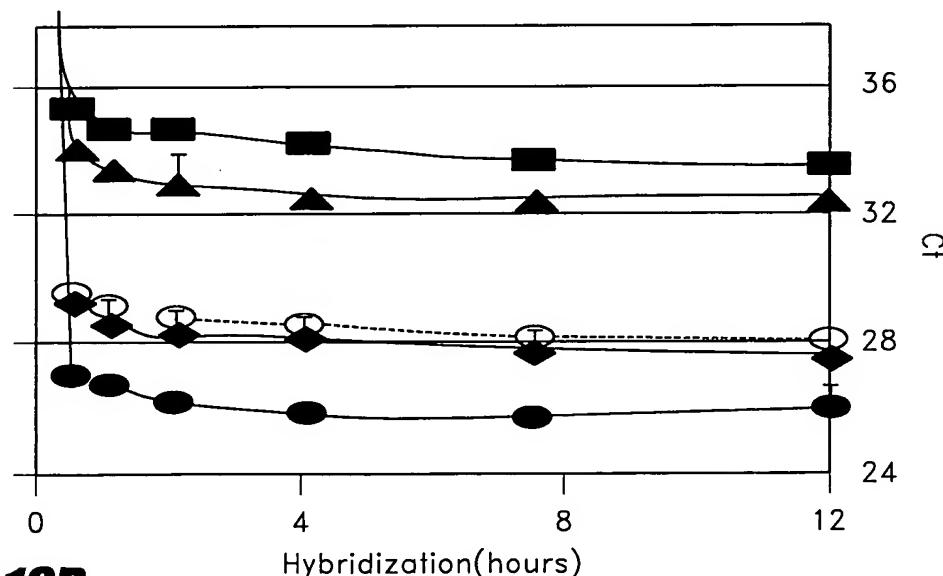


FIG. 19D

PCR cycles.

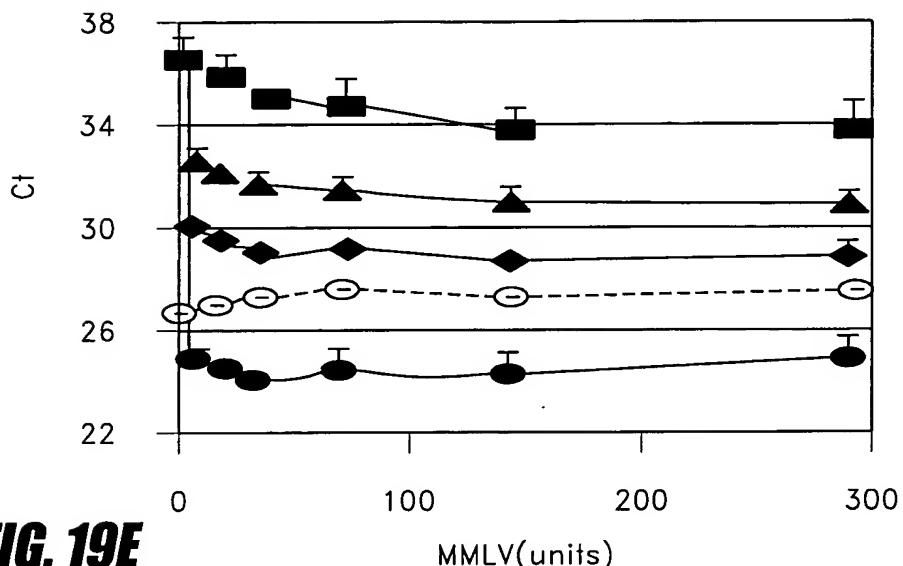


FIG. 19E

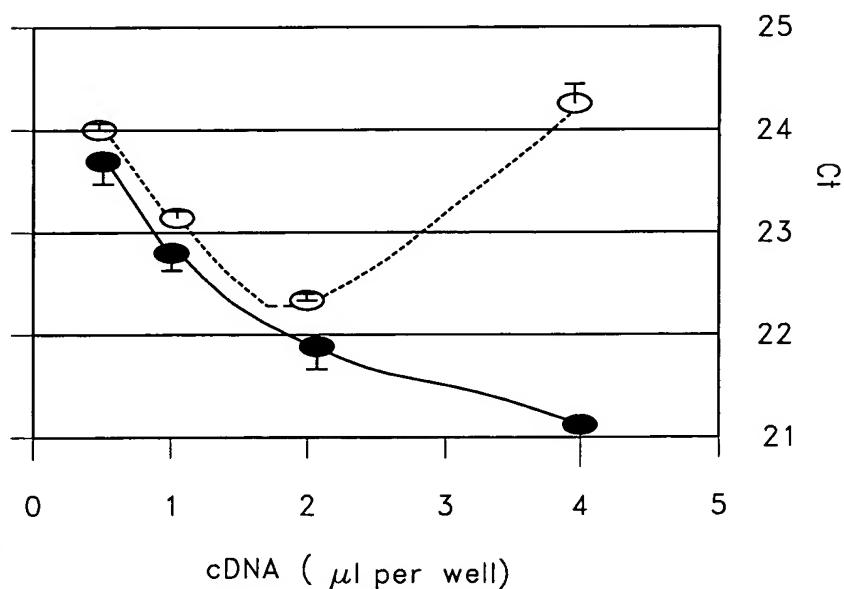


FIG. 19F

Performance of Control RNA.

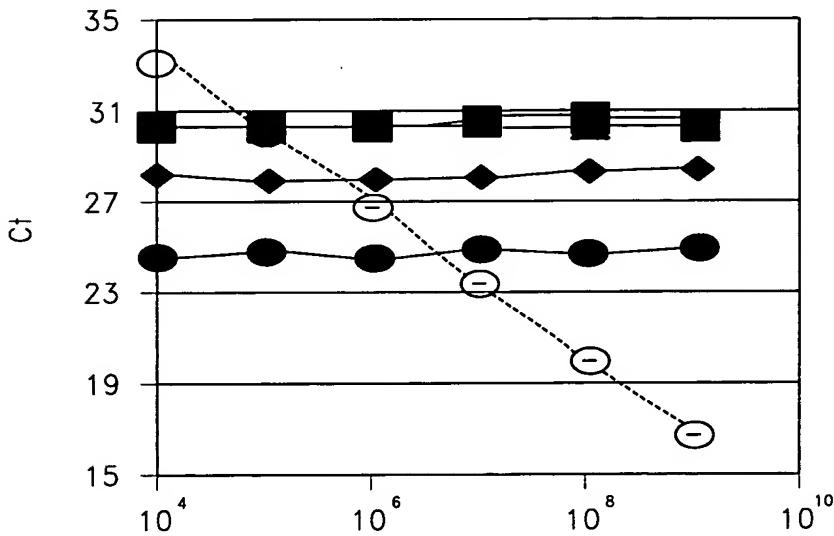


FIG. 20A Spiked standard RNA(molecules per well)

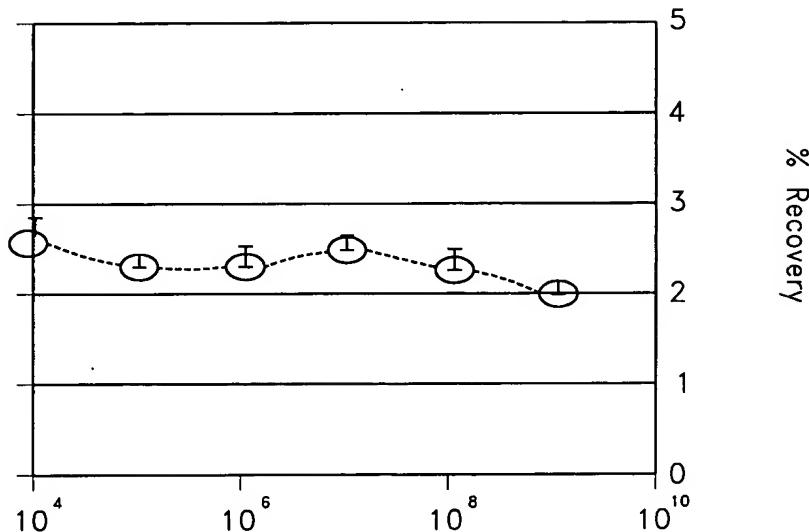


FIG. 20B

Spiked standard RNA(molecules per well)

Performance of Control RNA

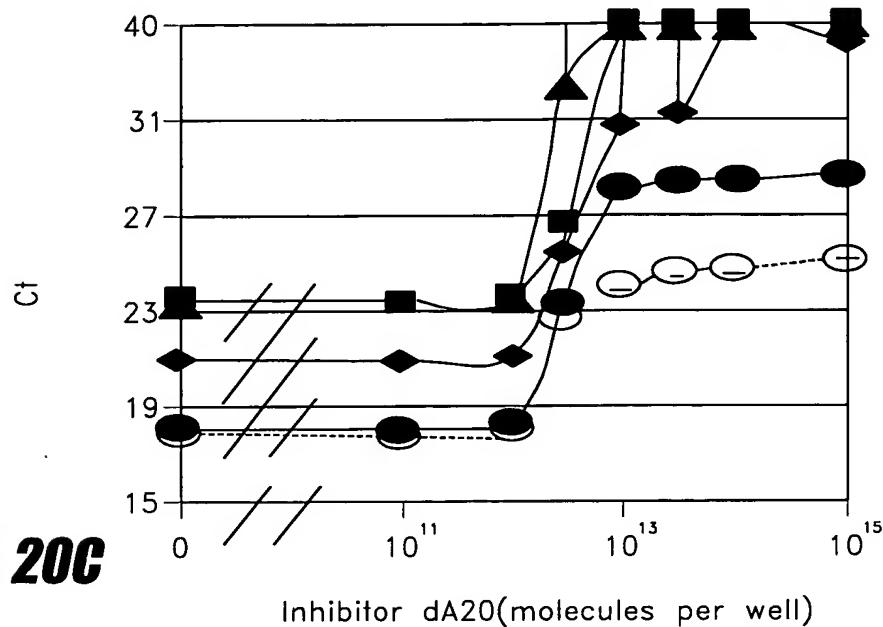


FIG. 20C

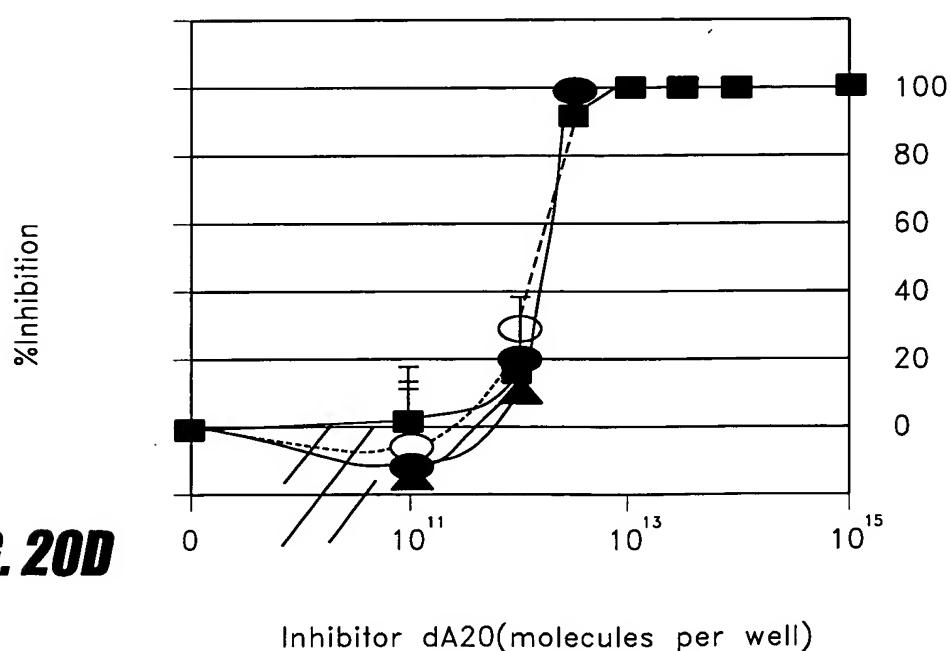


FIG. 20D

Performance of Control RNA

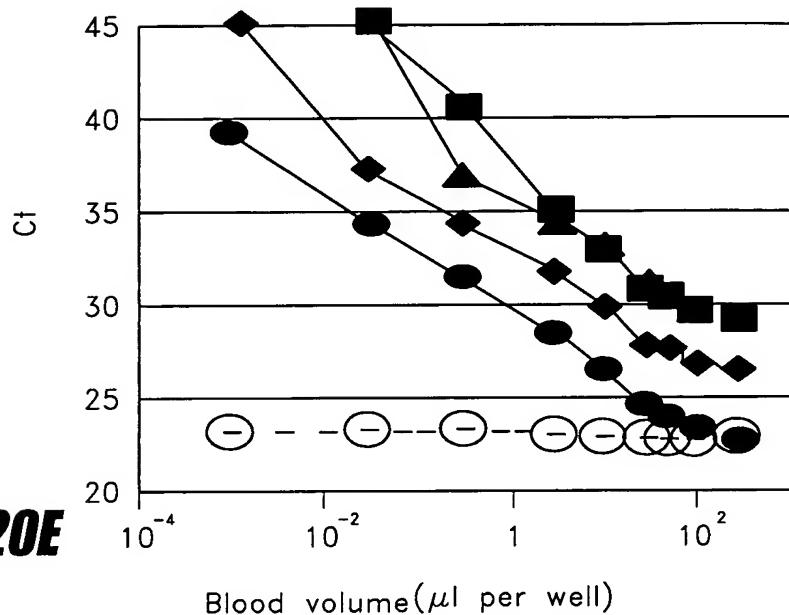


FIG. 20E

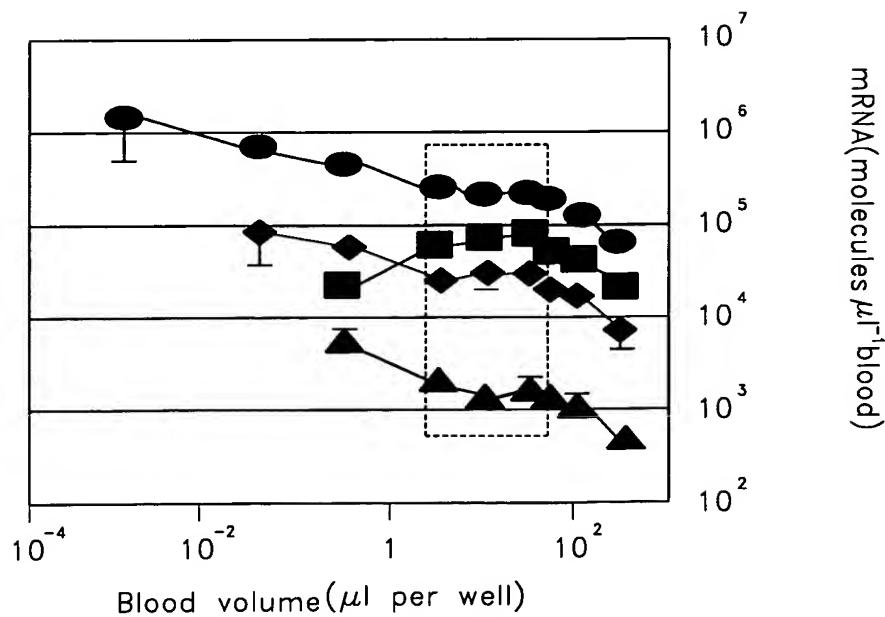


FIG. 20F

mRNA recovery of various subjects.

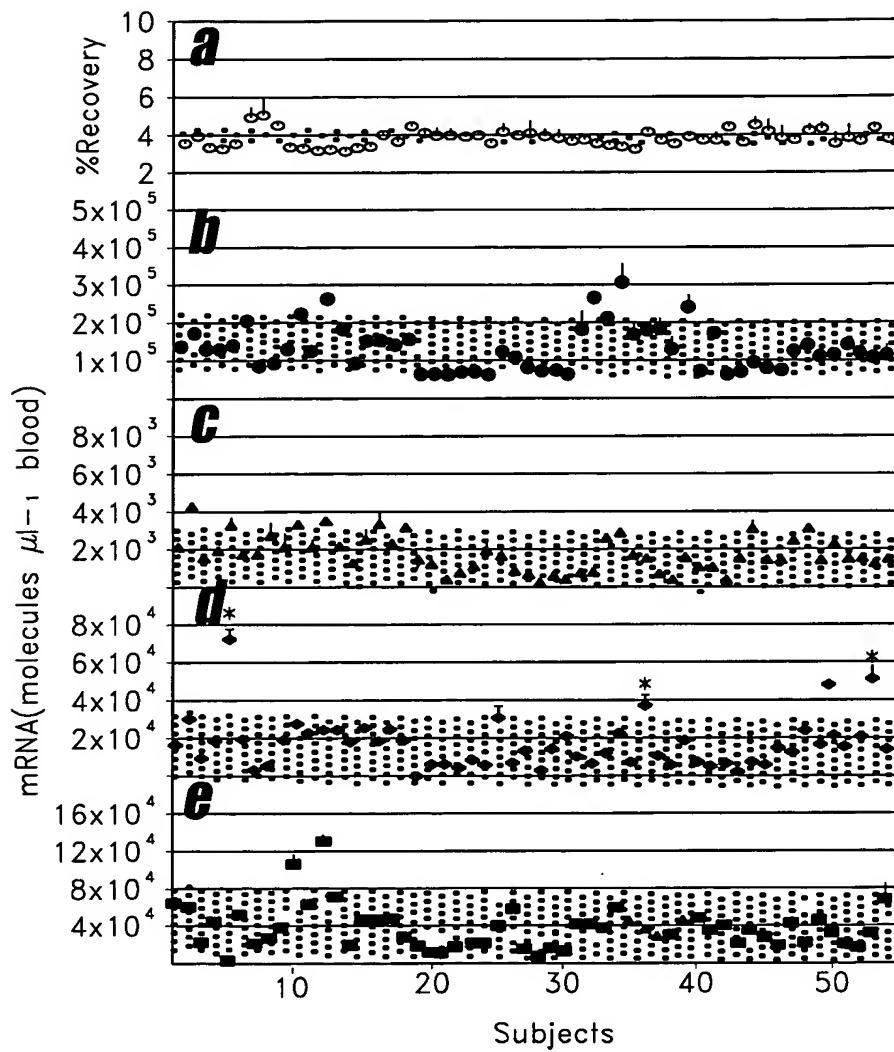


FIG. 21A-21E

Recovery of mRNA among various subjects.

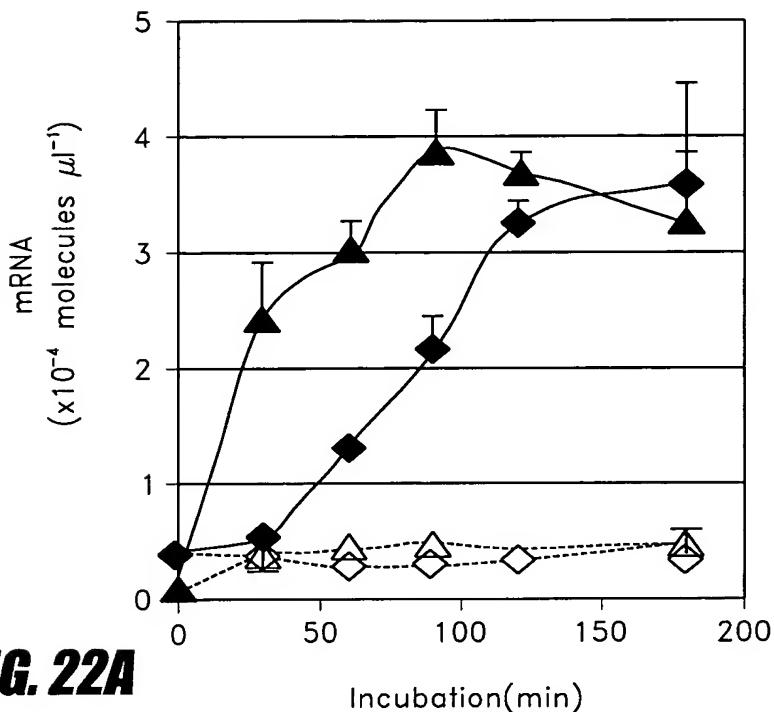


FIG. 22A

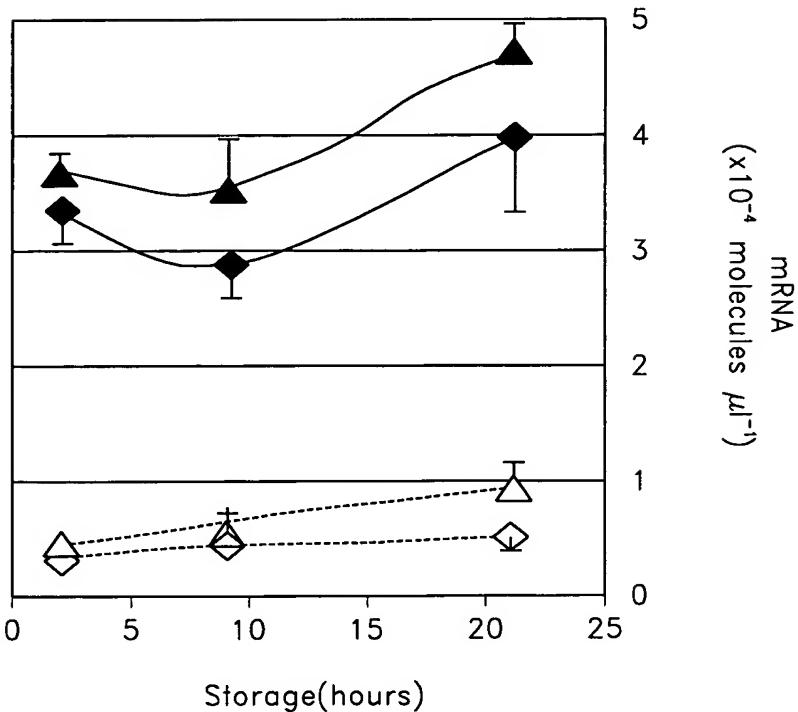


FIG. 22B

Recovery of RNA among various subjects

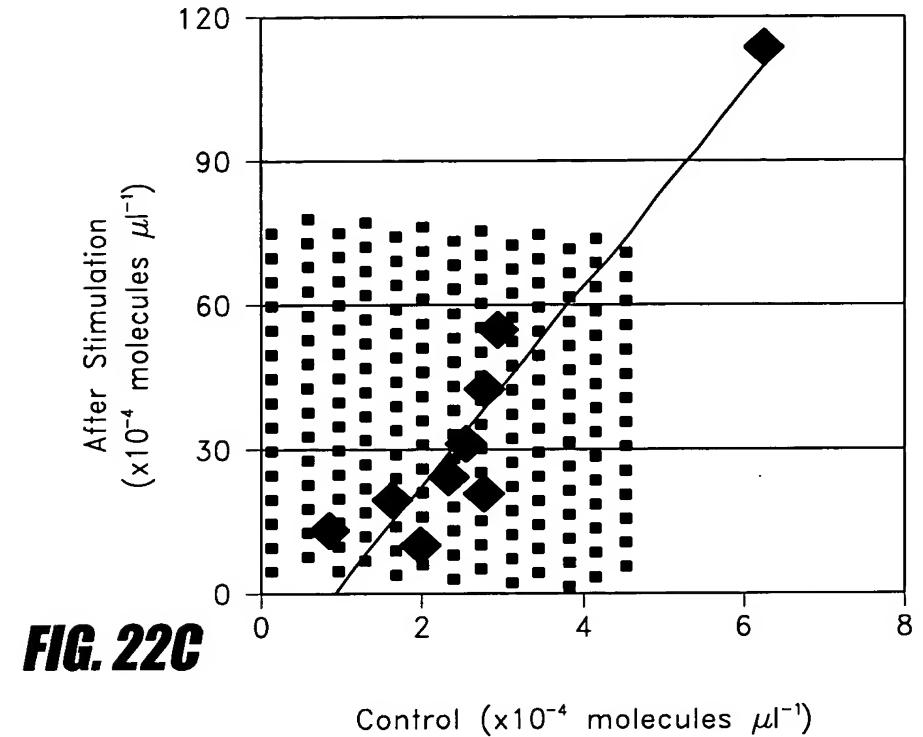


FIG. 22C

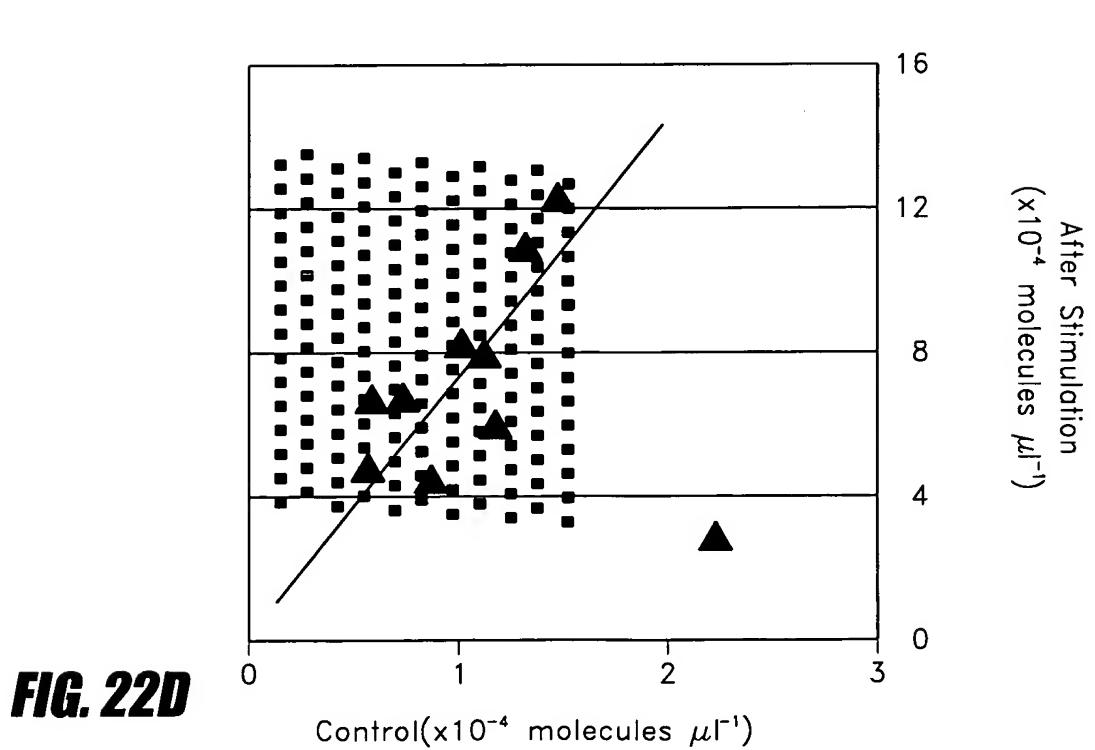


FIG. 22D

Recovery of mRNA various subjects

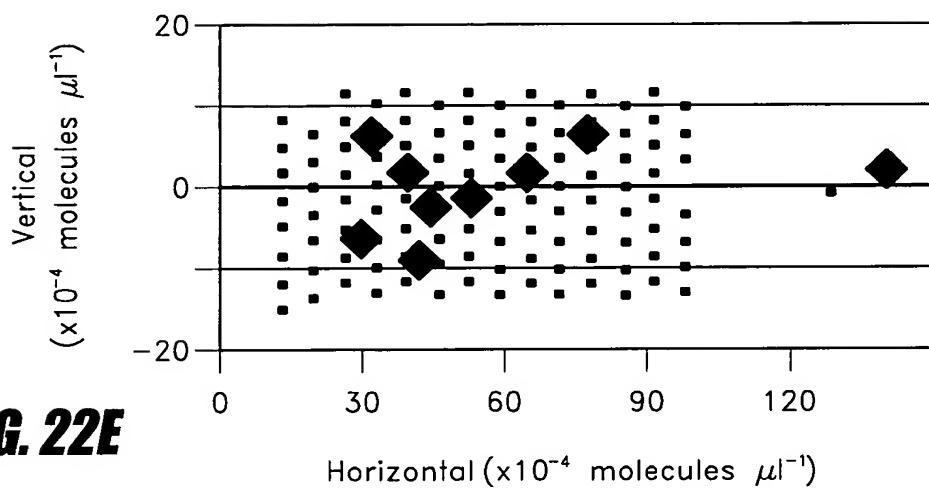


FIG. 22E

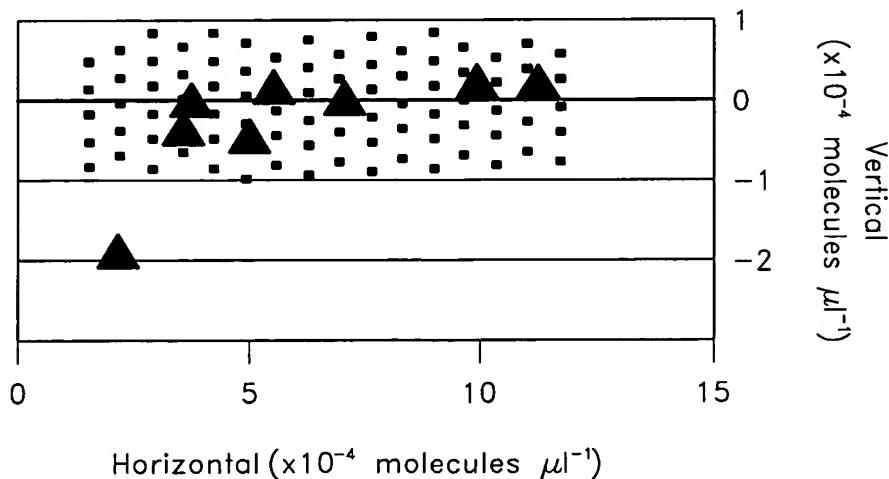


FIG. 22F

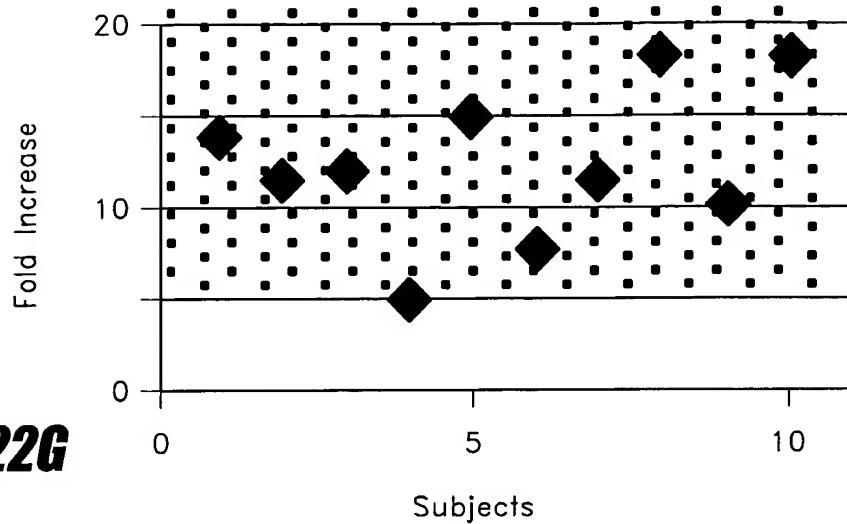


FIG. 22G

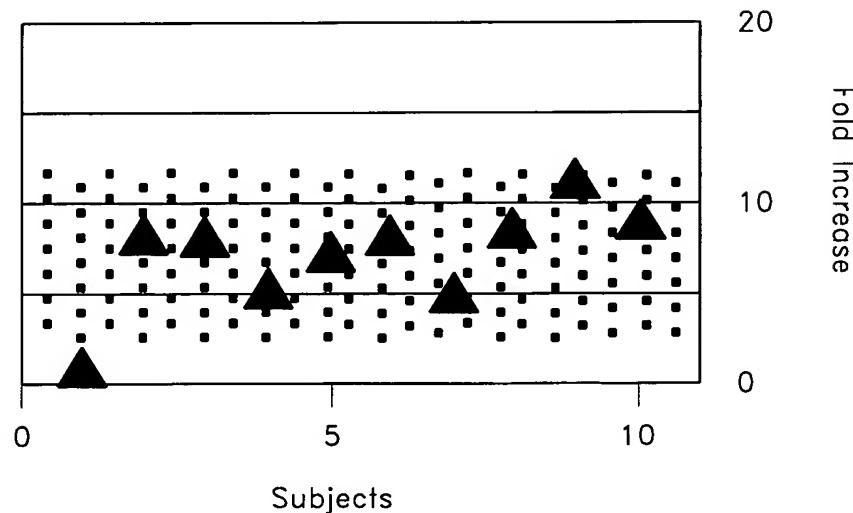


FIG. 22H